METHOD AND AGENTS FOR INHIBITING PROTEIN AGING

This invention was made in part with government support under Grant Number PHS AM 19655 awarded by the National Institutes of Health. The government has certain rights in the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of U.S. Ser. No. 220,504, filed Jul. 18, 1988, now abandoned, which is a division of U.S. Ser. No. 798,032 filed Nov. 14, 1985 and now U.S. Pat. No. 4,758,583, which is a continuation-in-part of U.S. Ser. No. 590,820, filed Mar. 19, 1984 and now U.S. Pat. No. 4,665,192. Applicants claim the benefits of these Applications under 35 U.S.C. §120.

RELATED PUBLICATIONS

The Applicants are co-authors of the following articles directed to the subject matter of the present invention: "COVALENT ATTACHMENT OF SOLUBLE PROTEINS BY NONENZYMATICALLY GLYCOSYLATED COLLAGEN; ROLE IN THE IN SITU FORMATION OF IMMUNE COMPLEXES, Brownlee, M., Pongor, S., Cerami, A., *J. Exp. Med.*, 158, pp. 1739-1744 (1983); "AGING OF PROTEINS: ISOLATION AND IDENTIFICATION OF A FLUORESCENT CHROMOPHORE FROM THE REACTION OF POLYPEPTIDES WITH GLUCOSE", Pongor, S., Ulrich, P., Bencsath, A. A., and Cerami, A., *Proc. Natl. Acad. Sci. USA*, 81, pp. 2682-2688 (1984); and "ADVANCED GLYCOSYLATION END PRODUCTS IN TISSUE AND THE BIOCHEMICAL BASIS OF DIABETIC COMPLICATIONS", Brownlee, M., Cerami, A., and Vlassara, H., *The New England Journal of Medicine*, 318, pp. 1315-1321 (1988). All of the above publications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates generally to the aging of proteins resulting from reaction of glucose, and particularly to the nonenzymatic glycosylation of proteins and subsequent reactions leading to advanced glycosylation end products, and to methods and agents for their inhibition.

The reaction between glucose and proteins has been known for some time. Its earliest manifestation was in the appearance of brown pigments during the cooking of food, which was identified by Maillard in 1912, who observed that glucose or other reducing sugars react with amino acids to form adducts that undergo a series of dehydrations and rearrangements to form stable brown pigments, Maillard, L.D. *C.R. Acad. Sci.*. 154, pp. 66-68 (1912).

In the years that followed the initial discovery by Maillard, food chemists studied the hypothesized reaction in detail and determined that stored and heat treated foods undergo nonenzymatic browning as a result of the reaction between glucose and the polypeptide chain, and that the proteins are resultingly crosslinked and correspondingly exhibit decreased bioavailability. At this point, it was determined that the pigments responsible for the development of the brown color that develops as a result of protein glycosylation possessed characteristic spectra and fluorescent properties; however, the chemical structure of the pigments had not been specifically elucidated.

The reaction between reducing sugars and food proteins discussed above was found in recent years to have its parallel in vivo. Thus, the nonenzymatic reaction between glucose and the free amino groups on proteins to form a stable amino, 1-deoxy ketosyl adduct, known as the Amadori product, has been shown to occur with hemoglobin, wherein a rearrangement of the amino terminal of the β-chain of hemoglobin by reaction with glucose, forms the adduct known as hemoglobin A_{1c}. The reaction has also been found to occur with a variety of other body proteins, such as lens crystallins, collagen and nerve proteins. See Bunn, H.F., Haney, D.N., Gabbay, K.H. and Gallop, P.H., *Biochem. Biophys. Res. Comm.*, 67, pp. 103-109 (1975); Koenig, R.J., Blobstein, S.H. and Cerami, A., *J. Biol. Chem.*, 252. pp. 2992-2997 (1975); Monnier, V.M. and Cerami, A. in *Maillard Reaction in Food and Nutrition*, ed. Waller, G.A., American Chemical Society, 215, pp. 431-448 (1983); and Monnier, V.M. and Cerami, A., *Clinics in Endocrinology and Metabolism*, 11, pp. 431-452 (1982).

Moreover, brown pigments with spectral and fluorescent properties similar to those of late-stage Maillard products have also been observed in vivo in association with several long-lived proteins, such as lens proteins and collagen from aged individuals. An age-related linear increase in pigment was observed in human dura collagen between the ages of 20 to 90 years. See Monnier, V.M. and Cerami, A., *Science*, 211, pp. 491-493 (1981); Monnier, V.M. and Cerami, A., *Biochem. Biophys. Acta.* 760, pp. 97-103 (1983); and Monnier, V.M., Kohn, R.R. and Cerami, A., "Accelerated Age-Related Browning of Human Collagen in Diabetes Mellitus", *Proc. Natl. Acad. Sci.*, 81, pp. 583-587 (1984). Interestingly, the aging of collagen can be mimicked in vitro by the crosslinking induced by glucose; and the capture of other proteins and the formation of adducts by collagen, also noted, is theorized to occur by a crosslinking reaction, and is believed to account for the observed accumulation of albumin and antibodies in kidney basement membrane. See Brownlee, M., Pongor, S. and Cerami, A., *J. Exp. Med.*, 158, pp. 1739-1744 (1983); and Kohn, R. R., Cerami, A. and Monnier, V.M., *Diabetes*, 33(1), pp. 57-59 (1984).

Recently, the role of other naturally-occurring reducing sugars, including fructose, in nonenzymatic crosslinking has been discussed. Specifically, Suarez et al. "Administration of an Aldose Reductase Inhibitor Induces a Decrease of Collagen Fluorescence in Diabetic Rats", *J. Clin. Invest.*, 82, pp. 624-627 (1988) have shown that fructose levels are elevated in diabetes as a result of the elevated glucose being channeled through the polyol pathway, first to sorbitol then to fructose. These investigators also showed that the ability of fructose to cause nonenzymatic crosslinking as measured by collagen fluorescence, is 10 times greater than that of glucose. Because the methods and agents of the present invention block nonenzymatic crosslinking mediated by any of the reactive sugars, they are expected to prevent fructose-mediated crosslinking as well. Cross-linking caused by other reactive sugars present in vivo or in foodstuffs, including ribose and galactose, would also be prevented by the methods and compositions of the present invention.

In parent application Ser. No. 590,820 (now U.S. Pat. No. 4,665,192) and in Pongor, S.M., et al., supra, both incorporated herein by reference, a fluorescent chromo-